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EXPERIMENTAL THERAPEUTICS

Exploring the Pharmacokinetic/Pharmacodynamic Relationship of Relebactam (MK-7655) in Combination with Imipenem in a Hollow-Fiber Infection Model

Jin Wu,[a] Fred Racine,[a] Michael K. Wismer,[a] Katherine Young,[a] Donna M. Carr,[a] Jing C. Xiao,[a] Ravi Katwaru,[a] Qian Si,[a] Paul Harradine,[a] Mary Motyl,[a] Pratik R. Bhagunde,[a] Matthew L. Rizk[a]

[a]Merck & Co., Inc., Kenilworth, New Jersey, USA

ABSTRACT Resistance to antibiotics among bacterial pathogens is rapidly spreading, and therapeutic options against multidrug-resistant bacteria are limited. There is an urgent need for new drugs, especially those that can circumvent the broad array of resistance pathways that bacteria have evolved. In this study, we assessed the pharmacokinetic/pharmacodynamic relationship of the novel β-lactamase inhibitor relebactam (REL; MK-7655) in a hollow-fiber infection model. REL is intended for use with the carbapenem β-lactam antibiotic imipenem for the treatment of Gram-negative bacterial infections. In this study, we used an *in vitro* hollow-fiber infection model to confirm the efficacy of human exposures associated with the phase 2 doses (imipenem at 500 mg plus REL at 125 or 250 mg administered intravenously every 6 h as a 30-min infusion) against imipenem-resistant strains of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Dose fractionation experiments confirmed that the pharmacokinetic parameter that best correlated with REL activity is the area under the concentration-time curve, consistent with findings in a murine pharmacokinetic/pharmacodynamic model. Determination of the pharmacokinetic/pharmacodynamic relationship between β-lactam antibiotics and β-lactamase inhibitors is complex, as there is an interdependence between their respective exposure-response relationships. Here, we show that this interdependence could be captured by treating the MIC of imipenem as dynamic: it changes with time, and this change is directly related to REL levels. For the strains tested, the percentage of the dosing interval time that the concentration remains above the dynamic MIC for imipenem was maintained at the carbapenem target of 30 to 40%, required for maximum efficacy, for imipenem at 500 mg plus REL at 250 mg.

KEYWORDS β-lactamase inhibitor, antibiotic resistance, hollow-fiber model, pharmacokinetic/pharmacodynamic, relebactam

The β-lactam antibiotics, especially the carbapenem group of antibiotics, have long been the silver bullet against bacterial infections. Since their discovery, the use of these antibiotics has drastically reduced mortality and increased life expectancy (1). However, the emergence of resistance to commonly used antibiotics poses a significant threat to the treatment of bacterial infections. The rise in resistance to carbapenems, normally used as a last resort against multidrug-resistant bacteria, is particularly alarming (2, 3). One of the primary causes of resistance to β-lactam antibiotics, including carbapenems, is the production of β-lactamase enzymes (4). Among various strategies, the most successful in increasing the life span of β-lactam antibiotics has been their combination with new agents capable of inhibiting a broad spectrum of β-lactamases (4, 5). Relebactam (REL; MK-7655) is a β-lactamase inhibitor that inhibits two different classes of β-lactamases: class A β-lactamases (serine-containing β-lactamases, such as the *Klebsiella pneumoniae* carbapenemase) and class C β-lactamases (such as AmpC cephalosporinases) (6, 7). *In vitro* and *in vivo* studies show that REL effectively restores the

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Address correspondence to Jin Wu, jin_wu@merck.com.

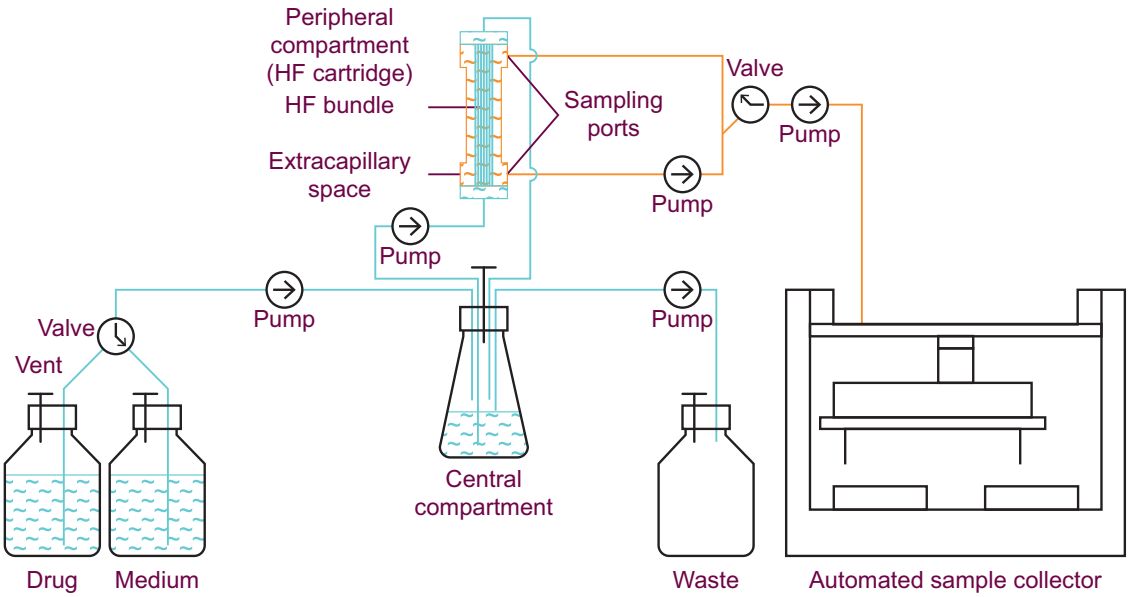

FIG 1 Schematic of the two-compartment *in vitro* hollow-fiber system. Forward arrow, liquid flow direction; continuous lines, physical connections; HF, hollow fiber.

activity of imipenem against imipenem-resistant *Pseudomonas aeruginosa* and *K. pneumoniae* strains (6–9).

Extensive pharmacokinetic (PK)/pharmacodynamic (PD) information from *in vitro* and *in vivo* studies, coupled with multiple-dose safety and PK data from phase 1 studies, supported phase 2 dose selection of imipenem at 500 mg plus REL at 125 or 250 mg administered intravenously every 6 h (q6h) (10). However, additional information is needed to assess the key PK/PD drivers of REL pharmacology when dosed in combination with imipenem.

PK/PD studies are important to bridge the preclinical data to ensure adequate dose selection for clinical trials. Furthermore, determination of these parameters for antimicrobials is vital to select breakpoints and determine combination therapies (11). However, as some β-lactamase inhibitors, including REL, do not have direct antimicrobial activity (12), it is challenging to determine the PK/PD relationship between β-lactam antibiotics and β-lactamase inhibitors. Mathematical modeling of *in vitro* data allows for a comprehensive evaluation of various dosing strategies for drug-inhibitor combinations, while it reduces the requirement for experimental studies in animals (7, 13, 14). Such modeling, when coupled with studies using *in vitro* PD models, can complement the knowledge gained from *in vivo* studies in animal models (15–17).

Here, we report on the use of a semiautomated hollow-fiber infection model (18) to study the PK/PD of the imipenem-REL combination in clinical isolates of *P. aeruginosa* and *K. pneumoniae*. The hollow-fiber infection model has been used extensively to determine the PK/PD relationship for various drugs against many infectious pathogens (16, 19). In this study, we also coupled the results from the hollow-fiber model with those from mathematical modeling of the time-varying MIC to study a number of different combinations of imipenem-REL and to understand comprehensively the PK/PD relationship of this combination.

RESULTS

PK simulation. Imipenem and REL free-drug concentrations in the extracapillary space of the hollow-fiber cartridges (Fig. 1) were similar to the expected human PK (free-drug concentrations) (Fig. 2A and B). These results indicate the accurate setup of the *in vitro* system and the computer software controlling the system.

Strains of *P. aeruginosa* for testing in the hollow-fiber system were obtained from the Merck Clinical Culture Collection or the IHMA freezer collection and selected on the

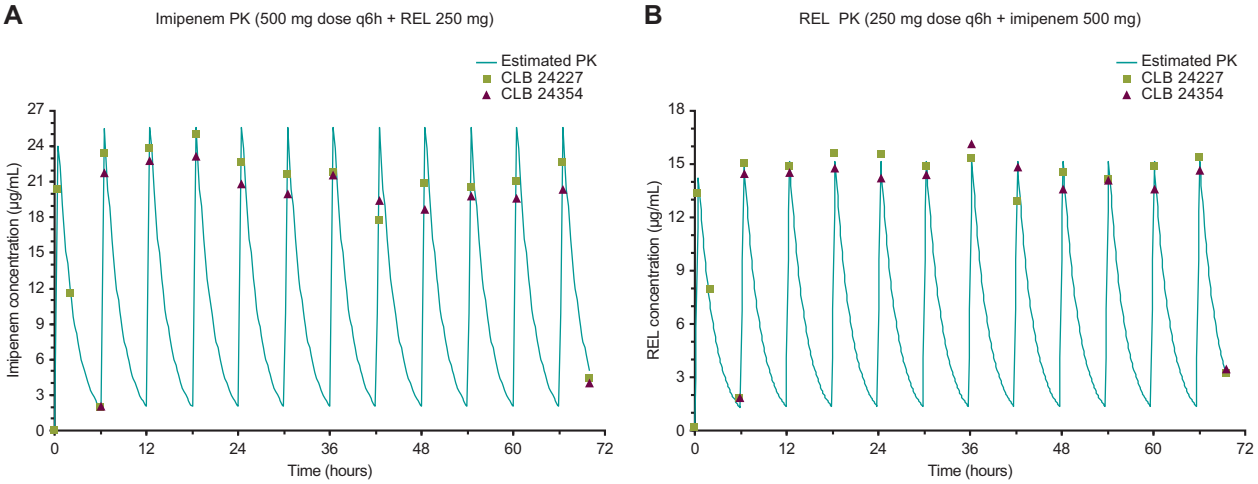

FIG 2 Simulated target and observed concentrations of imipenem (A) and REL (B) in the hollow-fiber system. CLB 24227, *Pseudomonas aeruginosa* isolate CLB 24227; CLB 24354, *Pseudomonas aeruginosa* isolate CLB 24354; PK, pharmacokinetic; q6h, every 6 h; REL, relebactam.

basis of the MIC of imipenem. It is well understood that the susceptibility of *P. aeruginosa* to imipenem depends on both the loss of functional OprD (the entry porin for imipenem) and high levels of AmpC (the chromosomal AmpC β-lactamase encoded by the bla_{PDC} gene) (20). AmpC may be constitutively produced or induced, and imipenem is itself an inducer of AmpC. Thus, in the presence of imipenem, maximal expression of the enzyme is observed. However, the amount of AmpC production is a property of the individual isolate. For the isolates listed in Table 1, a relationship of the level of induced β-lactamase to the imipenem MIC may be observed, with isolates with the highest induced expression having the highest imipenem MIC and those with the lowest induced expression having the lowest imipenem MIC. A correlation between the intrinsic imipenem MIC and the imipenem MIC in the presence of the *in vitro* susceptibility concentration of REL of 4 μg/liter is also seen. Note that no apparent relationship of the PDC allele to the imipenem MIC or the imipenem-REL MIC was observed.

TABLE 1 Strains evaluated in the hollow-fiber system for imipenem-REL

Organism	Isolate	Imipenem MIC (μg/ml)	Imipenem MIC (μg/ml) in presence of REL at 4 μg/ml	β-Lactamase gene or β-lactamase produced[a]	β-Lactamase activity[b]	
					Basal level	Inducible level
Pseudomonas aeruginosa	CLB 24226	32	4	bla_{PDC-19}	8,198	11,019
Pseudomonas aeruginosa	CLB 24227	16	2	bla_{PDC-35}	502	6,827
Pseudomonas aeruginosa	CLB 24228	32	8	bla_{PDC-16}	160	5,598
Pseudomonas aeruginosa	CLB 24354	64	16	bla_{PDC-3}	14,602	12,306
Pseudomonas aeruginosa	CL 5701	16	2	bla_{PDC-5}	3,880	5,304
Klebsiella pneumoniae	CL 6339	64	1	KPC-2, TEM, SHV	NA	NA
Klebsiella pneumoniae	CL 6569	256	4	KPC-2	NA	NA
Klebsiella pneumoniae	CLB 26410	>256	8	KPC-2	NA	NA
Klebsiella pneumoniae	CL 5763	32	0.5	KPC-3	NA	NA
Klebsiella pneumoniae	CL 6838	16	0.5	KPC-3	NA	NA
Klebsiella pneumoniae	IHMA 516426	16	2	CTX-M-14, DHA	NA	NA
Serratia marcescens	IHMA 1203541	16	16	CTX-M-3	NA	NA
Escherichia coli	IHMA 1231530	4	1	CTX-M-15, CMY-2	NA	NA
Escherichia coli	IHMA 1224137	8	≤0.5	TEM-OSBL, CTX-M-14, CMY-140	NA	NA
Klebsiella oxytoca	IHMA 1211369	32	1	KPC-6	NA	NA
Klebsiella pneumoniae	IHMA 520284	8	1	KPC-11	NA	NA

[a]bla_{PDC}, chromosomally encoded AmpC (class C); KPC, class A serine *Klebsiella pneumoniae* carbapenemase; TEM, *Temoneira* β-lactamase; CTX-M, cefotaxime-hydrolyzing capability β-lactamase; CMY, cephamycin β-lactamase; DHA, Dhahran Hospital; SHV, sulfhydryl variable β-lactamase.
[b]Basal and induced β-lactamase activity is expressed in nanomoles of nitrocefin hydrolyzed per minute per milligram of protein. NA, not applicable; REL, relebactam.

PD against *P. aeruginosa* and *K. pneumoniae* strains. The growth of *P. aeruginosa* and *K. pneumoniae* strains with or without imipenem-REL was monitored using the automated sample collector. The time-kill data collected over a 72-h period are shown in Fig. 3. Both doses of REL showed rapid and sustained bactericidal activity against the *P. aeruginosa* strains CLB 24226, CLB 24227, and CLB 24228 (Fig. 3A to C). Against these strains, the number of bacterial CFU was below the detectable limit of 10 CFU/ml in just over 15 to 30 h, and a 4-fold log reduction versus the starting inoculum was observed (Fig. 3A to C). These three strains of *P. aeruginosa* had imipenem unpotentiated MIC values of 16 to 32 μg/ml (Table 1). In contrast, when the two imipenem-REL combinations were tested against the *P. aeruginosa* strain CLB 24354, which had a high level of resistance to imipenem (MIC, 64 μg/ml), the lower dose of REL (125 mg) did not prove to be efficacious and it took a longer time (>50 h) for REL at 250 mg to reduce the number of CFU to below detectable limits (Fig. 3D). Imipenem in combination with both doses of REL showed rapid and sustained bactericidal activity against carbapenemase-producing *K. pneumoniae* strain CL 6339 (imipenem MIC, 64 μg/ml), with a greater than 5-fold log reduction in the number of CFU occurring within 6 to 12 h of administration of the dose (Fig. 3E). Additional studies were conducted with 10 *Enterobacteriaceae* producing various class A and/or C enzymes. These studies were conducted only at the simulated phase 3 clinical dose of REL (250 mg). The isolates displayed various responses with imipenem alone at 500 mg. Imipenem plus REL at 250 mg was efficacious against all of these *Enterobacteriaceae*, with the reductions in the numbers of CFU being below the limit of detection and no grow-back occurring (Fig. 3F to O).

Influence of REL on MIC of imipenem. A total of 485 data points from 93 strains of *P. aeruginosa* were obtained from checkerboard experiments. In these experiments, the MIC for each strain was determined in the absence of REL and in the presence of increasing concentrations of REL, with 4 to 5 different concentrations of REL being tested against each strain, resulting in a robust data set to describe the relationship between the REL concentration and the imipenem MIC. The data indicated that the imipenem MIC of a strain changes in a concentration-dependent manner with increasing levels of REL and could be effectively captured in a mathematical framework utilizing a maximum-effect (E_{max}) model to describe the relationship between the potentiated MIC and the REL concentration (Fig. 4).

Percentage of the dosing interval that the imipenem-REL concentration remains above the dynamic MIC. At exposures associated with a REL dose of 200 mg, the percentage of the dosing interval that the imipenem-REL concentration remained above the dynamic MIC ($T>MIC_{dynamic}$) with an imipenem dose of 200 mg (less than half of the clinically administered dose of 500 mg) was approximately 40%, which is the level previously described as being required for efficacy when the MIC is treated as static (21, 22), against the *P. aeruginosa* CLB 24227 strain (Fig. 5A). The clinically administered dose of imipenem at 500 mg with REL at 250 mg demonstrated a $T>MIC_{dynamic}$ of 65.5%, which is well above the levels required for efficacy (Fig. 5A). Against *P. aeruginosa* strain CL 5701 (Fig. 5B), imipenem at 200 mg plus REL at 200 mg exhibited maximal efficacy, while achieving a percentage of the dosing interval that the concentration remained above the MIC ($T_{>MIC}$) of about 50%. Similar efficacy was observed when the concentrations were increased to simulate imipenem at 500 mg and REL at 250 mg, where the $T_{>MIC}$ was 66% (Fig. 5B). Together, these results suggest that incorporation of β-lactamase inhibitor pharmacology via a dynamic MIC is one method that allows PK/PD assessments to bridge to well-established targets for carbapenems when dosed as monotherapy. Further, the established threshold of a $T_{>MIC}$ of 40% is exceeded at the phase 3 clinical dose of imipenem at 500 mg plus REL at 250 mg.

Analysis of REL PK/PD using conventional PK/PD indices. A more conventional PK/PD analysis for REL is shown in Fig. 6A to H, where the relationship between the efficacy in the hollow-fiber infection model and various PK/PD indices, such as area under the concentration-time curve (AUC), the ratio of the AUC to the MIC (AUC/MIC),

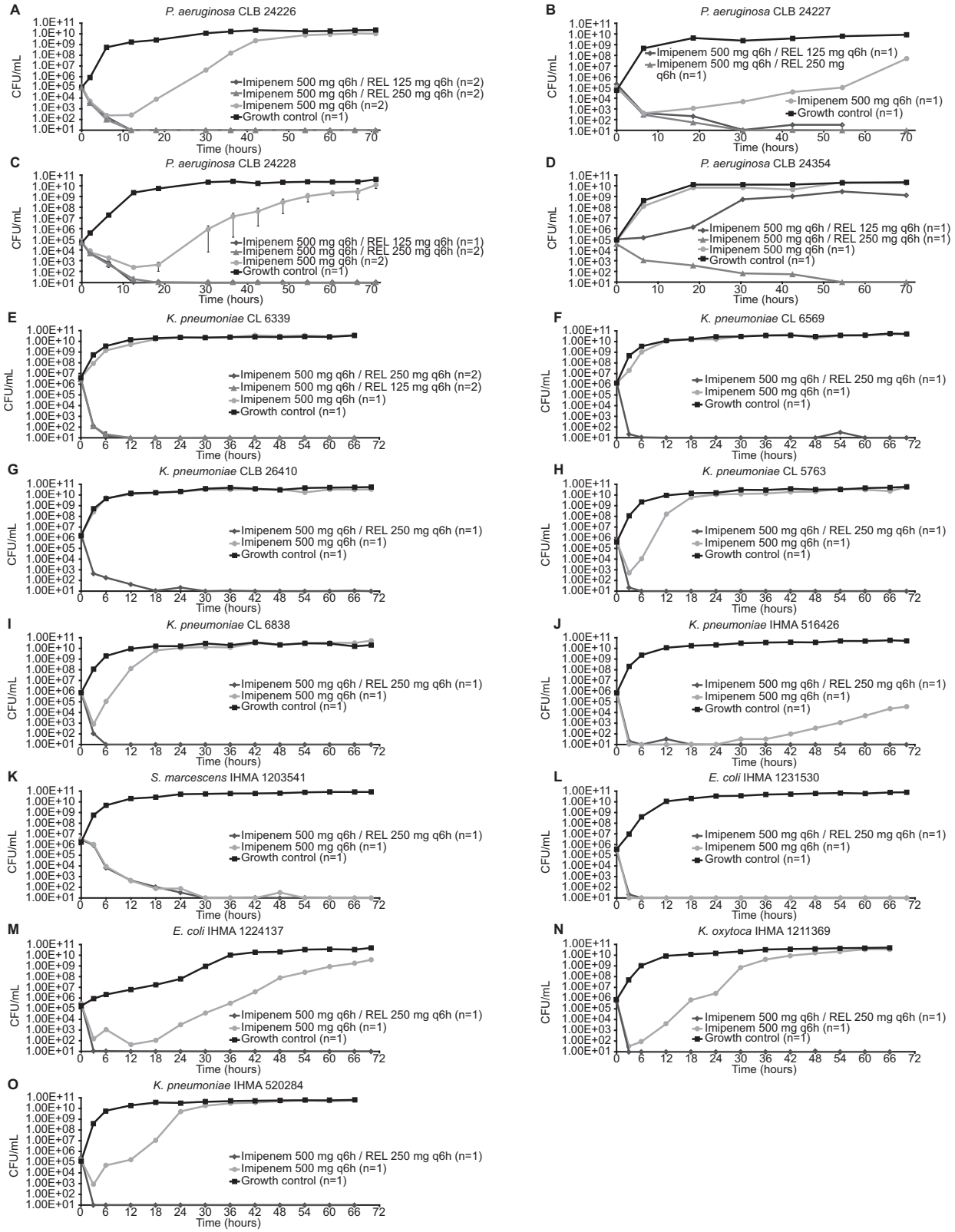

FIG 3 Microbiological responses in the hollow-fiber system for imipenem at 500 mg alone, imipenem at 500 mg q6h plus REL at 125 mg q6h, and imipenem at 500 mg q6h plus REL at 250 mg q6h against the following imipenem-resistant strains: *P. aeruginosa* CLB 24226 (A), *P. aeruginosa* CLB 24227 (B), *P. aeruginosa* CLB 24228 (C), *P. aeruginosa* CLB 24354 (D), *K. pneumoniae* CL 6339 (E), *K. pneumoniae* CL 6569 (F), *K. pneumoniae* CLB 26410 (G), *K. pneumoniae* CL 5763 (H), *K. pneumoniae* CL 6838 (I), *K. pneumoniae* IHMA 516426 (J), *Serratia marcescens* IHMA 1203541 (K), *Escherichia coli* IHMA 1231530 (L), *E. coli* IHMA 1224137 (M), *Klebsiella oxytoca* IHMA 1211369 (N), and *K. pneumoniae* IHMA 520284 (O). q6h, every 6 h; REL, relebactam.

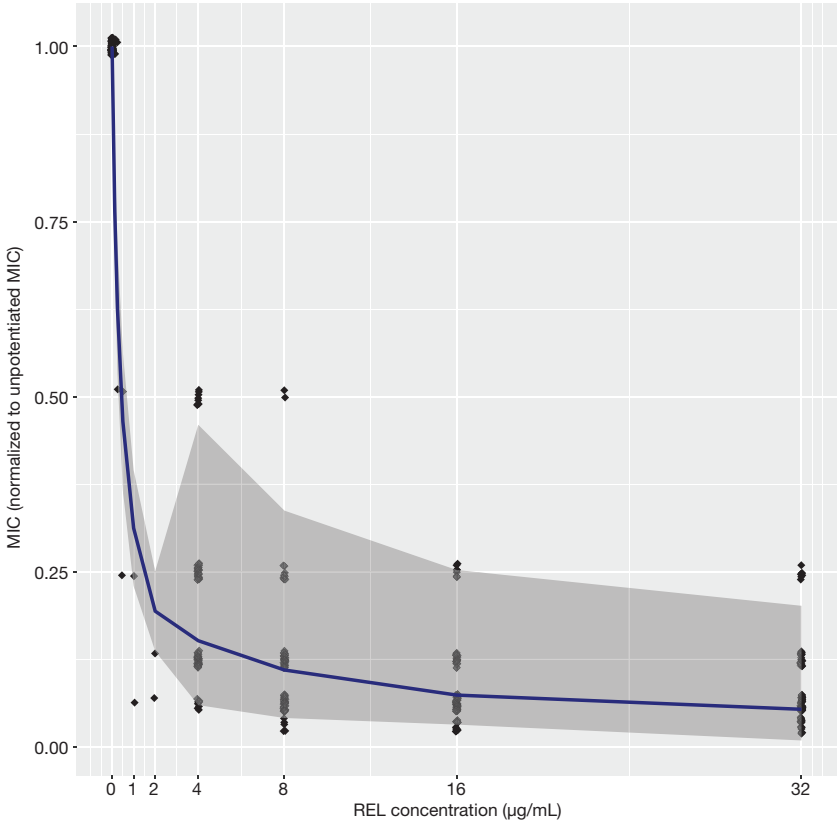

FIG 4 Relationship between the imipenem MIC and the REL concentration in static checkerboard experiments from a total of 485 data points from 93 strains of *P. aeruginosa* overlaid with the model fit drug concentration at which the maximum effect is observed (E_{max}) (symbols, experimental data; blue line, median model fit; shaded area, minimum-maximum model fit). REL, relebactam.

the maximum concentration (C_{max}), the ratio of C_{max} to the MIC (C_{max}/MIC), and the duration of the dosing interval that the concentration remains above the threshold concentration ($T_{>CT}$) with different threshold concentrations is shown. For the purpose of this PK/PD analysis, an array of hollow-fiber dose-ranging and dose-fractionation studies was used. The REL dosing regimens in various studies used for PK/PD analysis are shown in Table 2. The actual PK observed in each of the studies was used to compute the PK/PD indices, such as AUC, C_{max}, and $T_{>CT}$. AUC/MIC showed the best relationship with the efficacy observed in the hollow-fiber model when the coefficient of determination (r^2) for the model fit between the observed and model-predicted drop in log CFU at 48 h and the percent relative standard error on the model drug concentration at which half of the maximum effect was observed (EC_{50}) (Table 3) were considered. On the basis of the AUC/MIC relationship, the AUC/MIC ratios required to achieve static, 1-log, and 2-log drops in the hollow-fiber system were 8.2 h, 12 h, and 18 h, respectively. In this analysis, the MIC used to normalize PK parameters was the potentiated MIC (the MIC of imipenem in the presence of REL at 4 μg/ml).

DISCUSSION

Both phase 2 doses (imipenem at 500 mg plus REL 125 or 250 mg q6h) showed excellent efficacy against multiple imipenem-resistant *P. aeruginosa* and *K. pneumoniae* strains. This is consistent with the efficacy noted in other publications (21, 22). The only exception was the highly resistant *P. aeruginosa* strain CLB 24354 (MIC = 64 μg/ml). This strain exhibited the only observed dose response between REL doses of 125 mg and 250 mg. Imipenem exhibited maximal antibacterial activity against the strain only when used in combination with the higher REL dose. Furthermore, even at the higher dose, it took longer for the log reduction in the number of CFU of strain CLB 24354 to reach

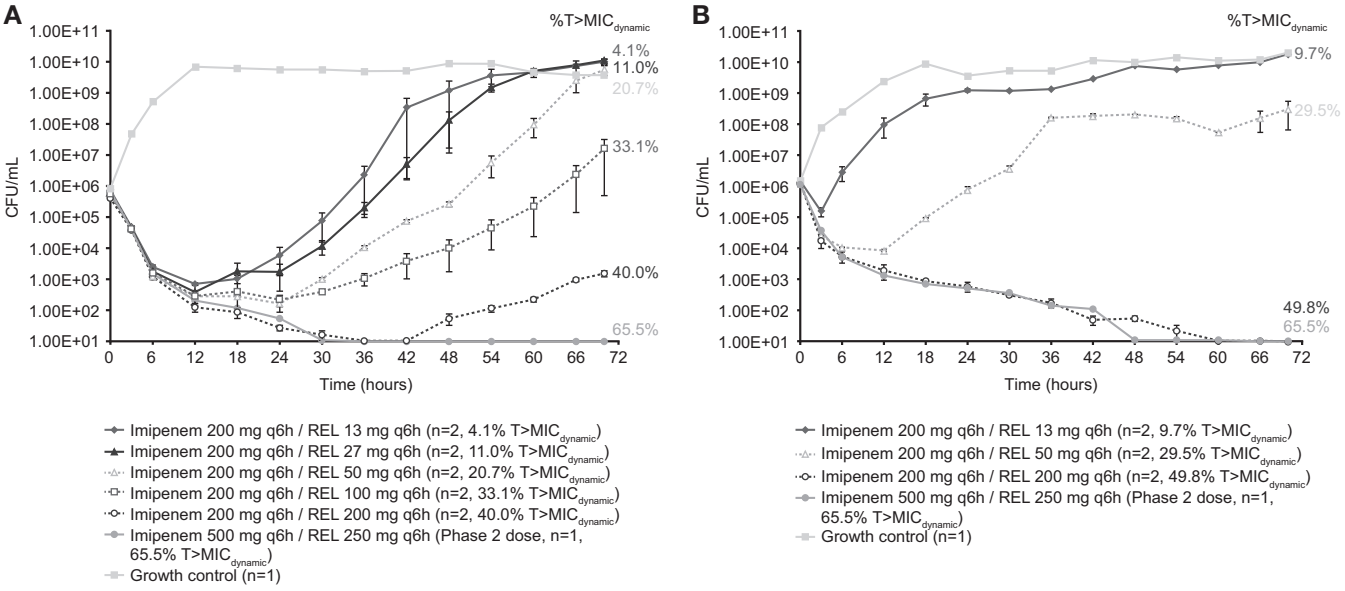

FIG 5 Microbiologic responses in the hollow-fiber system for various dose combinations of imipenem and REL against the following imipenem-resistant strains: *P. aeruginosa* CLB 24227 (A) and *P. aeruginosa* CL 5701 (B). q6h, every 6 h; REL, relebactam; $T{>}MIC_{dynamic}$, percentage of the dosing interval that the concentration remains above the dynamic MIC.

levels below detectable limits than it did for the other strains. This highly resistant strain requires 16 μg/ml of REL to reduce the imipenem MIC to the Clinical and Laboratory Standards Institute (CLSI) breakpoint; the clinical dose of imipenem plus REL was, therefore, not expected to demonstrate efficacy against this strain, as the required 16 μg/ml REL is 4-fold above the susceptibility testing concentration of 4 μg/ml utilized for REL.

As β-lactamase inhibitors generally do not have an antibacterial effect (12), the PK/PD index of a β-lactam–β-lactamase inhibitor combination is difficult to determine. According to the guidelines specified by the European Medicines Agency (23), a PK/PD index is to be established for each β-lactamase inhibitor in combination with the β-lactam antibiotic of interest. This index may vary according to the organism and the β-lactamase that it produces. In the case of β-lactam–β-lactamase inhibitor combinations, *in vitro* PD models have proven to be particularly valuable, as they facilitate experiments with a large number of different combinations of β-lactam and β-lactamase inhibitor dose regimens, allowing derivation of nonclinical PK/PD indices for the β-lactamase inhibitor (7, 18). The *in vitro* hollow-fiber infection model allows for flexible variation in drug concentration-time profiles and provides a relatively high-throughput and unconstrained way to explore the PK/PD relationship, significantly reducing the requirement for *in vivo* PK/PD studies in animals. For the current study, use of the hollow-fiber infection model saved approximately 2,000 mice. Furthermore, hollow-fiber infection models are an established method of studying PK/PD parameters and have been successfully used for a number of different drugs (16, 19, 24).

A more traditional way of discerning the β-lactamase inhibitor PK/PD relationship would be to use the three most common indices in antibiotic PK/PD, namely, $T_{>CT}$, C_{max}/MIC, and the ratio of the AUC from time zero to 24 h (AUC_{0-24}) to the MIC (AUC_{0-24}/MIC). The PK/PD index that best correlates with efficacy would be the driver for the β-lactamase inhibitor. With such an analysis, we found AUC/MIC to be best correlated with hollow-fiber infection model efficacy when all the hollow-fiber model data were pooled and analyzed together, as has also been determined in *in vivo* animal experiments conducted separately (25).

As mentioned before, the PK/PD relationship for β-lactam–β-lactamase inhibitor combinations is complex, with a high degree of interdependence between the

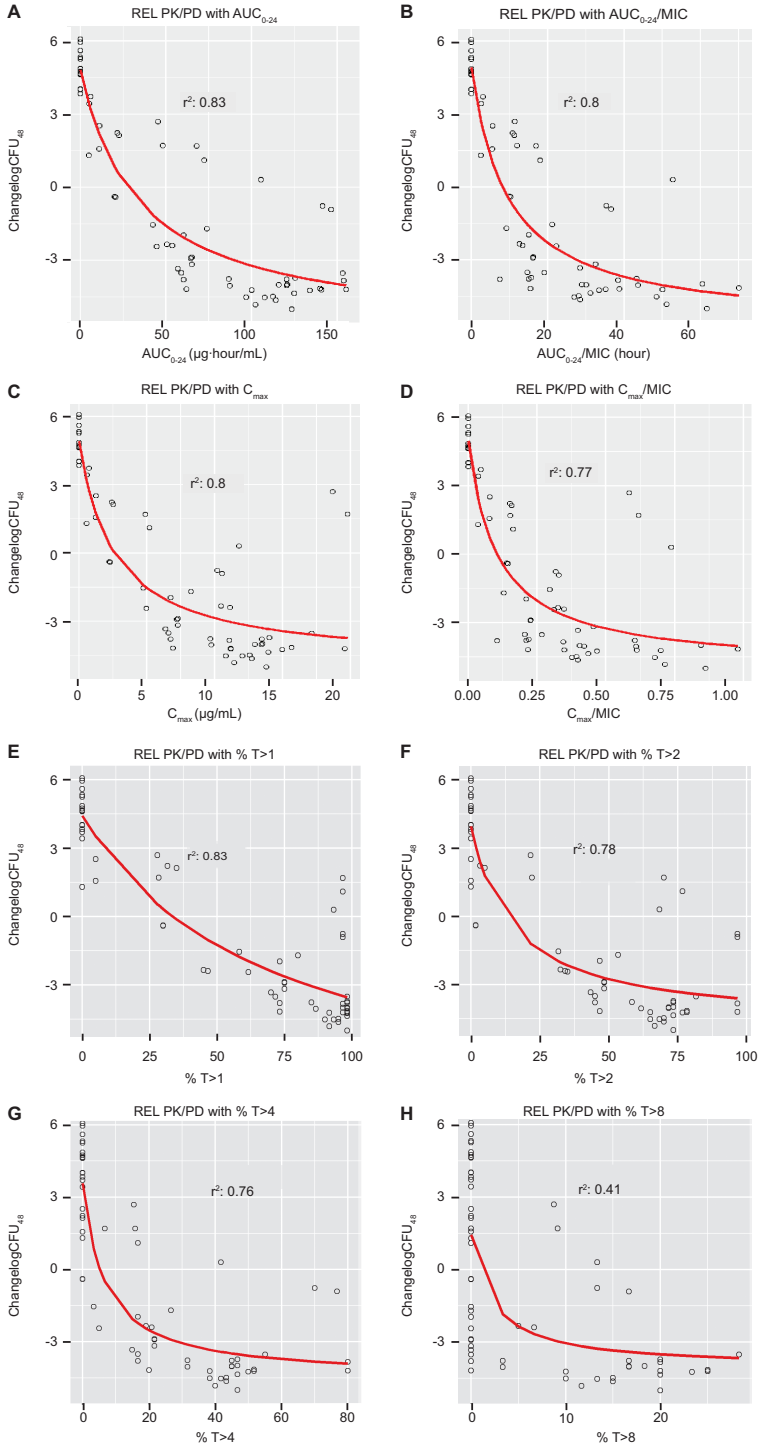

FIG 6 Relationship between 48-h log drop and different PK/PD indices: the area under the concentration-time curve from time zero to 24 h (AUC_{0-24}) (A), AUC_{0-24}/MIC (B), C_{max} (C), C_{max}/MIC (D), percent $T > 1$ (E), percent $T > 2$ (F), percent $T > 4$ (G), and percent $T > 8$ (H). Circles, experimental data; red line, median E_{max} model fit; $ChangelogCFU_{48}$, change in log CFU of bacteria in 48 h; percent $T > 1$, percentage of the dosing interval that the REL concentration is above the 1-μg/ml threshold; percent $T > 2$, percentage of the dosing interval that the REL concentration is above the 2-μg/ml threshold; percent $T > 4$, percentage of the dosing interval that the REL concentration is above the 4-μg/ml threshold; percent $T > 8$, percentage of the dosing interval that the REL concentration is above the 8-μg/ml threshold; AUC, area under the concentration-time curve; C_{max}, maximum concentration; PK/PD, pharmacokinetic/pharmacodynamic; r^2, coefficient of determination; REL, relebactam.

TABLE 2 REL dosing regimens studied in the hollow-fiber systems used for PK/PD analysis[a]

REL dose (mg)	Dosing interval (h)	Total daily dose (mg)
0		0
62.5	3	500
125	3	1,000
125	6	500
13	6	52
27	6	108
50	6	200
100	6	400
200	6	800
250	6	1,000
250	12	500
500	24	500

[a]PK/PD, pharmacokinetic/pharmacodynamic; REL, relebactam.

β-lactam and β-lactamase inhibitor concentrations existing. In order to assess this relationship appropriately, the target for the β-lactamase inhibitor needs to be established in a way that accounts for the β-lactam concentrations. Wild-type bacterial strains are susceptible to imipenem exposures with a $T_{>MIC}$ of 30 to 40% (26). A typical view of this system is that resistant strains produce β-lactamases, which hydrolyze imipenem. This would lead to a higher MIC and, subsequently, a lower $T_{>MIC}$ (Fig. 7A). Coexposure to REL would then restore the potency of imipenem by lowering the MIC in a concentration-dependent manner, and eventually, the MIC would be lowered to wild-type levels. In reality, on a local or microscopic level, the β-lactamases produced by resistant strains would result in lower local imipenem concentrations at the site of action, meaning that the MIC would not be reached (Fig. 7B). Coexposure to REL would then restore the potency of imipenem by inhibiting hydrolysis by β-lactamases, resulting in higher imipenem levels at the site of action. Since the microscopic or local imipenem levels cannot be directly assessed and changes in these levels are not reflected in the plasma PK that are typically measured, we instead adopted a formalism where (informed by checkerboard experimental data) the imipenem MIC of a strain changes in a concentration-dependent manner with time-dependent changes in the levels of REL (Fig. 7C). This dynamic or instantaneous MIC causes a fluctuation in susceptibilities to the β-lactamase. This model was first proposed by Bhagunde et al. to predict the *in vitro* activity of REL in combination with imipenem (13). This fluctuation in the MIC or the dynamic MIC is further affected by the efficiency and expression levels of the β-lactamase enzyme, which could have direct consequences on bacterial susceptibility and, in turn, would affect the intrinsic MIC. The key PK/PD parameter driving the efficacy of imipenem-REL would be $T>MIC_{dynamic}$ (13).

We further elaborated on the relationship between the MIC and the REL concentration by implementing the nonlinear mixed-effect model using the population approach, wherein each strain was considered an individual and the baseline MIC (MIC_0) for the strains was used. On the basis of these models, the imipenem-REL $T>MIC_{dynamic}$ was calculated for strains of *P. aeruginosa* in the hollow-fiber system. Imipenem at 200 mg q6h and REL at 200 mg q6h exhibited a $T_{>MIC}$ of 40%, and there was a significant reduction in the log density of the bacteria. Maximum efficacy was observed for imipenem at 500 mg q6h and REL at 250 mg q6h, wherein the $T_{>MIC}$ was 65.5%. This is consistent with studies of carbapenems that show that a $T_{>MIC}$ of ~40% is required for maximum efficacy (21, 22, 27). The correlation of our findings with existing observations shows that modeling of β-lactamase inhibitor PK/PD using a dynamic MIC will prove to be a useful tool in assessing such combination treatments in the future.

In summary, using an *in vitro* hollow-fiber infection model, we demonstrate that both phase 2 doses were effective against imipenem-resistant Gram-negative bacteria, with efficacy being related to the time above the dynamic MIC. At the time of this writing, REL

TABLE 3 EC_{50} estimate, percent relative standard error, and r^2 for different PK/PD models fit to the hollow-fiber data[a]

PK index	EC_{50} estimate	% RSE	r^2
AUC (μg · h/ml)	34.4	31.7	0.83
AUC/MIC (1/h)	9.60	29.3	0.80
C_{max} (μg/ml)	2.59	35.6	0.80
C_{max}/MIC	0.84	33.3	0.77
% $T > 1$ μg/ml	70	50.9	0.83
% $T > 2$ μg/ml	15	57.9	0.78
% $T > 4$ μg/ml	6.56	50.2	0.76
% $T > 8$ μg/ml	2.26	167	0.41

[a]% RSE, percent relative standard error; % $T > 1$, percentage of the dosing interval that the REL concentration is above the 1-μg/ml threshold; % $T > 2$, percentage of the dosing interval that the REL concentration is above the 2-μg/ml threshold; % $T > 4$, percentage of the dosing interval that the REL concentration is above the 4-μg/ml threshold; % $T > 8$, percentage of the dosing interval that the REL concentration is above the 8-μg/ml threshold; AUC, area under the concentration-time curve; C_{max}, maximum concentration; EC_{50}, drug concentration at which half of the maximum drug effect is observed; PK, pharmacokinetic; PD, pharmacodynamic; r^2, coefficient of determination.

has completed phase 2 clinical trials (ClinicalTrials.gov registration numbers NCT01505634 and NCT01506271) and is currently in phase 3 clinical trials (ClinicalTrials.gov registration numbers NCT02493764 and NCT02452047) studying a dose of imipenem at 500 mg plus REL at 250 mg administered intravenously every 6 h as a 30-min infusion.

MATERIALS AND METHODS

Antibiotics and bacterial strains. Imipenem and REL were dissolved in 3-(*N*-morpholino)-propanesulfonic acid (MOPS) and dimethyl sulfoxide (DMSO) at 10 mM, respectively, made to the appropriate concentrations needed, frozen at −70°C, and thawed immediately prior to use. Imipenem is extensively metabolized by a renal dipeptidase (dehydropeptidase I) present in the brush border of renal tubular epithelium in animal species and humans. To prevent its renal metabolism, imipenem is generally coadministered with cilastatin (a dehydropeptidase inhibitor) in animals and humans. However, there was no dehydropeptidase I present in the medium in the *in vitro* hollow-fiber studies described in the present report; therefore, cilastatin was not required. Imipenem was utilized alone or in combination with REL.

Sixteen isolates were evaluated in the hollow-fiber infection model. Five *P. aeruginosa* strains produced inducible or constitutive AmpC of various PDC alleles. *P. aeruginosa* and 11 *Enterobacteriaceae* isolates produced various alleles of KPC, CTX-M, TEM, CMY, and DHA, alone or in combination, as detailed in Table 1. All isolates were obtained from the Merck Clinical Culture Collection or the IHMA freezer collection of recent clinical isolates on the basis of previous molecular characterization (Table 1).

These strains were chosen to include strains with a range of imipenem MICs, either alone or in the presence of REL at 4 μg/ml. The *in vitro* susceptibility concentration of REL of 4 μg/ml was chosen on the basis of a phase 2 average concentration in patients of 4.94 μg/ml (10). Strains were grown from frozen glycerol stocks on Trypticase soy agar plates with 5% sheep blood (BD Laboratories, Franklin Lakes, NJ, USA), and susceptibility testing was performed in cation-adjusted Mueller-Hinton Broth (CAMHB). The MICs of imipenem were determined by the broth microdilution method, using the CLSI guidelines (28, 29), and all isolates were resistant to imipenem on the basis of CLSI breakpoints.

***In vitro* hollow-fiber model.** The two-compartment hollow-fiber model was adapted from models previously published by Grasso et al. (17) and Blaser et al. (15) and was developed as described earlier by Wang et al. (18). A schematic of the system is presented in Fig. 1.

The volume of distribution of the central compartment (V_c) was 150 ml, and imipenem and REL were added under computer control using a peristaltic pump. Fresh CAMHB was continuously supplied and removed from the central compartment via a peristaltic pump set to simulate the half-lives ($t_{1/2}$) of the antibiotics and the free fraction of drug in plasma (set at 1.5 h to replicate the clinical $t_{1/2}$ of imipenem and REL and the fraction of unbound drug [f_u] of 0.8 for both imipenem and REL). The flow rate was calculated to be 1.16 ml/min on the basis of the V_c and the $t_{1/2}$ per the equation $\ln 2 \cdot (V_c/t_{1/2})$.

The central compartment was incubated at 37°C with shaking at 80 rpm to ensure proper mixing of the drug. Samples of 0.2 ml were collected by the automated sample collector from the extracapillary space of the hollow-fiber cartridge (FiberCell Systems, Frederick, MD, USA) at 0, 3.5, 6.5, 12.5, 18.5, 24.5, 30.5, 36.5, 42.5, 48.5, 54.5, 60.5, 66.5, and 70 h for PK analysis.

For the PD evaluations, the initial challenge inoculum was prepared from overnight cultures grown on Trypticase soy agar plates with 5% sheep blood at 35°C. Colonies were picked from each overnight culture and suspended in 5 ml of Trypticase soy broth, and the turbidity was adjusted to match that of a 0.5 McFarland standard using a Siemens turbidity meter (preferred optical density, 0.10 to 0.12). This resulted in a concentration of approximately 1×10^8 CFU/ml. This standardized suspension was then diluted 1:1,000 into CAMHB and inoculated into the extracapillary space of the preconditioned hollow-fiber cartridges (FiberCell Systems, Frederick, MD, USA). Inoculated hollow-fiber cartridges were incubated at 37°C for 4 h prior to the start of the experiment; the resulting exponentially growing cultures reached ~10^6 CFU/ml. Samples of 0.25 ml were collected by the automated sample collector from the

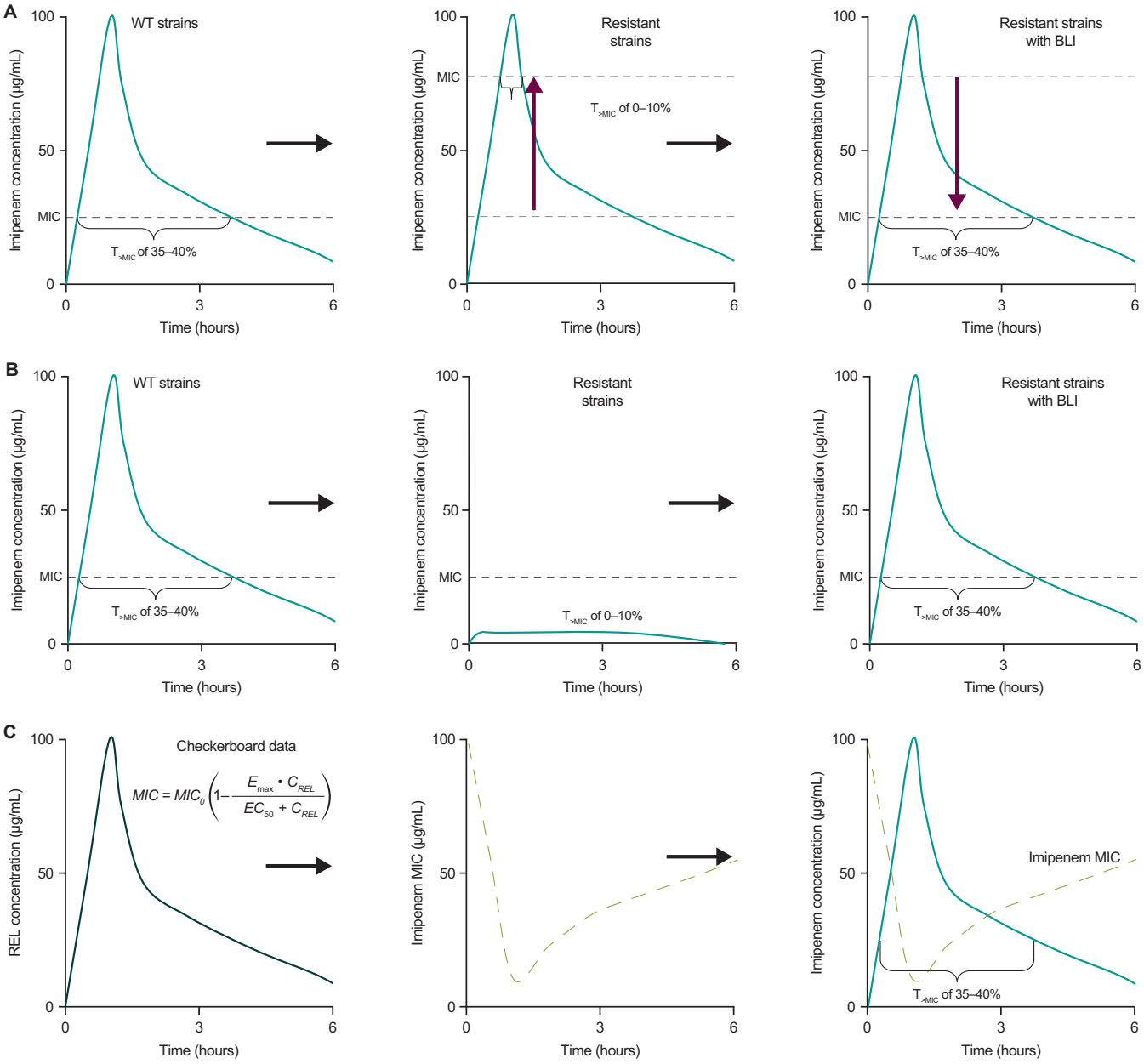

FIG 7 β-Lactam–β-lactamase inhibitor PK/PD relationship. (A) How we typically view the system in terms of resistance driving an increase in the MIC (increasing levels of resistance result in an increase in the MIC [middle]; coadministration with a β-lactamase inhibitor reduces the MIC [right]); (B) what actually occurs at the microscopic level at the bacterial membrane (β-lactamases hydrolyze the antibiotic, resulting in a lower level of drug at the target site [middle]; β-lactamase inhibitors prevent this hydrolysis, leading to higher drug levels at the site of action [right]); (C) formalism for calculation of a dynamic MIC of imipenem based on the REL PK profile (the MIC changes with time inversely to levels of the β-lactamase inhibitor [middle]; the target antibiotic $T_{>MIC}$ can be achieved when calculated relative to this time-changing MIC [right]). BLI, β-lactamase inhibitor; C_{REL}, concentration of relebactam; E_{max}, drug concentration at which the maximum effect is observed; EC_{50}, drug concentration at which half of the maximum drug effect is observed; MIC_0, baseline MIC; PK/PD, pharmacokinetic/pharmacodynamic; REL, relebactam; $T_{>MIC}$, percentage of the dosing interval that the concentration remains above the MIC; WT, wild type.

extracapillary space of the hollow-fiber cartridge at 0, 3, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, and 70 h for PD analysis.

Measurement of drug concentrations. Upon collection, samples were diluted 1:1 with a preservative composed of equal parts of ethylene glycol and a buffer [1 M 2-(*N*-morpholino)ethanesulfonic acid (MES), pH 6.0], which was prepared by adding MES (10.88 g; Sigma-Aldrich, St. Louis, MO, USA) and MES sodium salt (9.62 g) to 100 ml of water, mixing, and subsequently adding 100 ml of ethylene glycol. The stabilized samples were frozen at −80°C. Stable isotopically labeled internal standards of imipenem and REL were used for the high-performance liquid chromatography–mass spectrometry analysis. Sample preparation was performed over ice. CAMHB, preservative, acetonitrile, and a 10 mM MES buffer (pH 6.7) in acetonitrile/water (50/50, prepared by dilution of 1 M MES buffer, pH 6.7, 0.384 g MES, and 1.74 g MES

sodium salt in 10 ml water) were used in appropriate ratios during sample preparation to ensure matrix matching of the prepared unknown samples, standards, quality controls, as well as single and double blanks. A standard curve for concentrations ranging from 100 to 0.195 μg/ml was prepared. Protein precipitation was performed by the addition of a 5:1 ratio of acetonitrile to the matrix-matched samples. Following centrifugation and transfer of supernatant, samples were diluted 3-fold by the addition of 80% aqueous acetonitrile, chromatographed using hydrophilic interaction chromatography (HILIC), and detected via tandem mass spectrometry. Liquid chromatography was performed using a Waters HILIC (particle size, 3 μm; 2.1- by 50-mm column) held at 35°C, eluting isocratically with ammonium acetate 5 mM (pH 4.5) in acetonitrile/water (80/20) at a flow rate of 0.3 ml/min on a Thermo Fisher Scientific (Waltham, MA, USA) Transcend II multiplexed ultra-high-performance liquid chromatography system. An Applied Biosystems (Foster City, CA, USA) Sciex API 4000 triple-quadrupole tandem mass spectrometer equipped with an electrospray source was operated in positive-ion mode using multiple-reaction monitoring. The ion spray voltage was set to 4.5 kV, and the auxiliary gas temperature was maintained at 500°C. Nitrogen was used for gas 1, gas 2, curtain, and collision gas. The mass resolution was set to a peak width of 0.7 mass unit at half height for both quadrupole (Q)1 and Q3. The electron multiplier was set at 2,000 V.

Quantification of antibacterial effect. Bacterial viable counts were determined by serially diluting samples 10-fold in cold sterile saline. Aliquots of 100 μl of nondiluted and diluted samples (10^{-1} to 10^{-7}) were plated onto Trypticase soy agar plates with 5% sheep blood using an Eddy Jet spiral plater (Neutec Group Inc., Farmingdale, NY, USA) with subsequent incubation at 37°C for 18 to 24 h. Drug carryover was negligible after serial dilutions. After incubation, the resulting colonies were counted and the numbers of CFU per milliliter were determined using a Flash & Go colony counter (Neutec Group Inc., Farmingdale, NY, USA).

Data analysis. The simulated PK were captured with a one-compartment model fitted to the drug concentrations in the *in vitro* model central compartment (WinNonlin software; Pharsight Corp). The PK/PD relationship was analyzed by fitting a sigmoidal E_{max} relationship between the REL exposure (C_{max}, AUC, and $T_{>CT}$) and the associated response (antimicrobial effect, as measured by the change in bacterial counts). These relationships were assessed both by using the absolute value of the PK parameters C_{max} and AUC and also by normalizing the PK parameters to the potentiated MIC of the strain in the presence of 4 μg/ml REL. The time above the threshold concentration was explored using a range of concentration threshold values.

Analysis of REL PK/PD indices. REL PK/PD were assessed using the traditional indices frequently used in antibiotic PK/PD, namely, $T_{>CT}$, C_{max}, the C_{max}/MIC ratio, AUC_{0-24}, and the AUC_{0-24}/MIC ratio. The PK/PD index that best correlates with efficacy would be considered the driver for REL. In such a traditional analysis, different thresholds could be considered for the $T_{>CT}$ index. The relationship between efficacy and various indices is analyzed by a sigmoid E_{max} model. The model-predicted efficacy and observed efficacy at a specified time point are compared, in order to assess the best r^2. The index that provides the best r^2 is considered the driver for efficacy.

Influence of REL on the MIC of imipenem. The relationship between the MIC of imipenem and the REL concentration was captured by a nonlinear maximum-effect (E_{max}) model using a population approach, wherein each strain is considered an individual. The MIC_0 is the baseline, or unpotentiated, MIC for each strain. E_{max} is the maximum response to the drug (in this case REL), and EC_{50} is the drug concentration at which half of the maximum effect was observed. Populated parameters calculated for *P. aeruginosa* were E_{max} (0.96) and EC_{50} (0.6 μg/ml). Interstrain variability was accounted for as a random effect in the modeling. Checkerboard static exposure data from 93 strains of *P. aeruginosa* and the following equation were used to calculate the dynamic MIC of imipenem as a function of the time-varying concentration of REL: $MIC = MIC_0\{1 - [(E_{max} \cdot C_{REL})/(EC_{50} + C_{REL})]\} = MIC_0\{1 - [(0.96 \cdot C_{REL})/(0.6 + C_{REL})]\}$.

Percentage of the dosing interval that the imipenem-REL concentration remains above the dynamic MIC. The duration of the dosing interval that the imipenem concentration remains above the MIC is important for assessing the effectiveness of a dosing regimen. Therefore, the changing MIC or dynamic MIC against the *P. aeruginosa* and *K. pneumoniae* strains was studied in the hollow-fiber system using the same procedures and sample collection described earlier. To assess the REL PK driver, a subtherapeutic dose of imipenem of 200 mg q6h was applied in combination with a range of REL doses (13, 27, 50, 100, and 200 mg q6h). In addition, the phase 2B doses of imipenem at 500 mg plus REL at 125 mg or 250 mg q6h were also simulated.

Checkerboard methodology. All strains were streaked from frozen stocks onto Trypticase soy agar plates with 5% sheep blood on the day before use, and the plates were incubated at 37°C overnight. On the next day, 4 to 6 colonies from each plate were selected with a BD Prompt wand and resuspended in 1 ml sterile saline. The stock was estimated to be $\sim 1.5 \times 10^8$ CFU/ml. Bacterial stock solutions were diluted to 5.25×10^5 CFU per ml (15 ml CAMHB plus 52.5 μl bacterium-inoculated saline from the Prompt wand) to achieve a final starting concentration of bacteria of $\sim 5 \times 10^5$ CFU/ml when 95 μl of bacterial stock was added to the assay plate. Imipenem stock solutions were prepared in 2.5 mM MOPS, pH 7.4, and REL stocks were prepared in 100% DMSO. Twenty-times-titration plates were prepared, and 5 μl of the titration was transferred to replicate 96-well, round-bottom (clear) plates to achieve complete overlapping dilutions series of imipenem at concentrations ranging from 32 to 0.5 mg/ml and REL at concentrations ranging from 64 to 0.0625 mg/ml. Imipenem and REL were run in titration alone. Plates were shaken for 5 min on a rotary shaker and then incubated overnight (~19 h) at 37°C. On the next morning, the plates were read by eye for bacterial growth in the wells using a mirrored plate reader.

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